

THE IMPROVEMENT OF NATURALLY CROSS-  
POLLINATED PLANTS BY SELECTION  
IN SELF-FERTILIZED LINES

IV. Combining Ability of Successive Generations  
of  
Inbred Sweet Corn

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# THE IMPROVEMENT OF NATURALLY CROSS- POLLINATED PLANTS BY SELECTION IN SELF-FERTILIZED LINES

## IV. Combining Ability of Successive Generations of Inbred Sweet Corn<sup>1</sup>

W. RALPH SINGLETON AND OLIVER E. NELSON, JR.

Maize breeding has undergone a revolution in the past quarter of a century. Most of the sweet and field corn grown today is produced by crossing inbred strains which are comparatively pure lines. The method for producing this crossed seed is the one proposed by Shull, 1909, with modifications by Jones, 1920. By using this pure line method, the heredity of the hybrids can be controlled. This was not possible with any of the previous maize breeding methods. It is this controlled heredity that makes hybrid corn so superior to the open pollinated varieties.

Before crossing, it is necessary to secure inbred or pure lines of corn in order to have complete control over the hereditary composition and to obtain maximum hybrid vigor. This is usually done by self pollinating for three to eight generations before crossing. Thousands of inbreds have already been produced and the hand pollinations already made would be numbered in the millions. Many thousands are made each year.

The question naturally arose whether it might be possible to test the combining ability of inbreds in earlier generations of inbreeding than had been practiced previously, which was usually after an inbred had been selfed at least three times. Hence, it was decided ten years ago to undertake an experiment to determine the earliest possible generation of inbreeding in which selections could be made for combining ability. At that time, comparatively few data were available on this subject. Since this experiment was started, several papers have been published. It is worthwhile to review briefly the results of these experiments.

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<sup>1</sup>We wish to express our thanks to Dr. C. I. Bliss for suggesting the method of statistical analysis of the data presented here and for his assistance in the statistical analysis; also, to Dr. L. M. Roberts for help in compiling the data for the inbred plants.

## REVIEW OF LITERATURE

Jenkins (1935) tested the combining ability of inbreds in different generations of inbreeding, using 14 lines of Iodent and 14 of Lancaster Sure Crop, each having been selfed eight times. Remnant seed of each generation except the seventh was grown and crossed on an open pollinated variety, Krug, to produce top crosses for testing. Jenkins was also testing the effect on combining ability of selection within the inbred lines in different generations of inbreeding. He did this by taking at random in each generation a sister line to the one that had been selected and propagated. He found that selection between sister progenies was effective in isolating progenies whose crosses were slightly but consistently more productive than those of their discarded sibs. This point was not emphasized by Jenkins since he found greater differences between lines and suggested greater possibilities for progress in selection *among* rather than *within* lines. However, we believe the first finding of Jenkins, which has been corroborated by others, has an important bearing on breeding technique as will be pointed out later.

In regard to the generation of inbreeding in which selection for combining ability factors could be successfully practiced, Jenkins found the lines acquired their factors for combining ability very early in the inbreeding process and little change could be effected after the second generation of selfing. Regarding variability or uniformity of the hybrids, he found no differences between hybrids made with inbreds after successive generations of inbreeding. Since all of the inbreds were crossed by an open pollinated variety, Krug, all of the hybrids tested were top-crosses. Such crosses may be sufficiently variable for all generations of inbreeding to mask differences in uniformity of crosses made after successive generations of inbreeding.

Jones and Singleton (1935) found no benefit, as far as combining ability is concerned, in making new selections in the second generation of inbreds of Stowell's Evergreen sweet corn. None of the new hybrids from inbreds selected in the second generation of inbreeding was better than the hybrid made using the original C63 and 50 inbreds.

Jenkins (1940) studied the segregation for yielding ability of inbreds that had been selfed only once when crossed. He found significant differences between sibling plants within the same lines, but a higher difference between lines. These results supported his 1935 conclusions that there are greater possibilities for selection *among* large numbers of inbred lines than *within* lines. He also emphasized testing lines for combining ability in the early generations of inbreeding. Sprague and Bryan (1941) found a significant difference in combining ability for yield after three and four generations of selfing. Differences were larger among  $F_3$  lines. Hence, selection among  $F_3$  families might be more effective than among  $F_4$  lines. These results are different from those of Jenkins (1935). It is not surpris-

ing to find such a difference in two experiments and the need for more experimental evidence on this subject is emphasized. The experiment reported here gives additional evidence. Before setting forth this evidence, it may be well to review briefly some of the more important researches dealing with characters in the inbred parents that are more or less closely associated with yield of the  $F_1$  hybrids of such inbreds. Proper selection for such characters is an important part of any maize breeding program and must be utilized in conjunction with tests for combining ability of the inbreds.

Nilsson-Leissner (1927) and Jorgenson and Brewbaker (1927) in two separate experiments, showed, by means of multiple correlations, that length and diameter of ear, number of rows of kernels, height of plants and yield of inbred parents were positively correlated with yield of the  $F_1$  hybrids. Jorgenson and Brewbaker concluded that the "selection of the most vigorous selfed lines for the production of single and double crosses or synthetic varieties is the proper procedure for practical corn breeding". This recommendation was in complete agreement with that of Nilsson-Leissner. Jenkins (1929) corroborated these findings for height of plant, length and diameter of ear and yield of inbred ears. In addition, he found the following characters in the inbreds to be positively associated with yield of the  $F_1$  hybrids: date of tasseling and silking, number of nodes per plant and below ear, and number of ears per plant. Negative association was found for ear shape index (Diameter/Length).

Davis (1934) found a high and significant correlation between inbred-variety yields (top crosses) and the yields of the inbred parents in the first two generations of inbreeding ( $r = .638 \pm .08$ ). He concluded that average yield of the first two selfed generations could be safely used for elimination of some of the lines.

Jones and Singleton (1935) found that inbreds classified as good produced more than their share of the good hybrids. In a series of double crosses, yields were compared for double crosses having no, one, two and three good inbred strains as parents. The difference between no and three good factors was  $6 \pm 1.8$ . In a series of white flint crosses, there was a coefficient of association of 40 between inbreds selected and hybrids yielding better than the check. In a comparison of the hybrids of 74 inbred lines of Whipple's Early Yellow sweet corn, it was found that all but one of the good hybrids came from different combinations of nine selected inbreds. In this experiment, we could have discarded the remaining 65 inbreds before testing without losing the best hybrids. Since such rigid selection cannot be relied on to give all the good hybrids, it is not advocated as a policy.

Hayes and Johnson (1939) studied, for a three year period, correlation between 14 characters in the inbred lines and yield of the inbred-variety crosses. They found that 12 of these showed positive and significant correlations. These are: date silked, plant height, ear height, leaf area, pulling resistance, root volume, stalk diameter,



total brace roots, tassel index, pollen yield, yield index and ear length. Since these characters, which make for general vigor of the inbred parent, are positively associated with the combining ability of the inbred as measured by the yield of variety-inbred crosses, Hayes and Johnson made the following pithy conclusion: "*It would appear that the production of improved inbreds, as measured by the development of the inbreds themselves, will lead, on the average, to the development of higher yielding double crosses.*"

Hayes and Johnson also studied the combining ability of inbreds secured by the pedigree method of inbreeding first generation hybrids. They found that lines of good combining ability are obtained more frequently from crosses of inbreds that are good combiners than from crosses between inbreds that are low in combining ability. They concluded, therefore, that combining ability is an inherited character.

Summarizing the pertinent findings of these papers we can make the following generalization which we might call an axiom of maize breeding. *In a series of maize inbreds of common origin, the best lines, as measured by the appearance of their morphological characters or by the yield of the inbreds themselves, will produce, when crossed, their full share of the good hybrids.* This does not mean that the best hybrids will necessarily come from the very best inbreds. It does mean that the poorest inbreds can be safely discarded, knowing full well that the remaining inbreds will produce proportionately as many good hybrids as would have been produced had the whole lot of inbreds been tested. Knowledge and utilization of this principle is fundamental to any intelligent maize breeding program. This will be discussed more fully later in the bulletin.

## DESIGN OF EXPERIMENT

In the autumn of 1934, ears were selected from the seed barn of a farmer who was growing a seed field of Whipple's Early Yellow for the Comstock-Ferre Co. in Wethersfield, Connecticut. Particular attention was paid to securing as many different types of ears as possible. In all, 48 ears were chosen. Illustrations of the ears used are shown in Figure 1. Seed from these ears was planted in 1935 and one row was grown from each ear. One self-pollination was made from each lot and the pollen placed on several ears of Purdue 39, since it was desired to secure a midseason inbred that would produce a good market corn when crossed by P39. Preliminary observation rows of the 48 different hybrids were tested in 1936 and the better appearing hybrids noted. Also, in 1936, the 48 selfed lines of Whipples were grown, selfed again, and the pollen placed on several ears of P39. In 1937, the hybrids from both the 1935 and 1936 pollinations were grown and preliminary selections made. Likewise, in 1937, the lines were selfed again, making three generations selfed. In 1938, all four generations of the various lines, open pollinated, selfed once, twice and three times were grown in a crossing plot with Purdue 39 as the pollen





FIGURE 1. The 48 original open pollinated Whipple ears used in the investigation, 1-16, upper row; 17-32, center row, and 33-48, bottom row, numbering from left to right in each case.

parent. The purpose was to provide ample seed for three years testing. Not all 48 lines could be grown so 10 lines were selected for the further experiment. By that time, the original 48 lines had been reduced to 32 by losses or discarding of lines with poor plant characteristics. The 10 lines selected for the continuing experiment were chosen either because their hybrids had been selected in 1936 or 1937 or because the inbred itself was particularly good. Inbred 43 was chosen for this latter reason, although the hybrid with P39 had not been selected in either 1936 or 1937. We realize that in making the selection of the 10 lines in this manner, we are not taking a random sample of the inbreds and our results may, therefore, be biased in the direction of the good hybrids, if it is possible to select for combining ability as early as the O. P. single ear or the first generation selfed. However, this was a breeding, as well as a genetic, project and, in such experiments, we use every means to "stack the deck" in our favor. The results of this experiment will give some evidence as to how successful we were in selecting the better hybride on the basis of preliminary results of crossing the different inbreds by Purdue 39.

The generations of inbreeding that were grown in the 1938 crossing plots were as follows: O. P., representing seed from the original open pollinated ear; the A generation, representing one generation selfed, and the B and C lines, selfed two and three times, respectively. In designating the number of generations selfed throughout this bulletin, capital letters represent selfed lines, small letters, sibbed lines, and the position in the alphabet represents the number of generations of inbreeding. For example: A= selfed once, b= sibbed twice, C= selfed three times and so on.

Crossed seed of the four generations of the 10 lines of Whipples. 850-5, 8, 17, 25, 28, 30, 40, 41, 43 and 44 was produced in 1938 and crosses were tested in 1939 and 1940. In 1940, additional crossed seed was produced in a Purdue 39 crossing plot for testing in 1941. By this time, it was necessary to use seed of slightly different degrees of inbreeding since the seed of the original open pollinated ears was exhausted, as was the seed for some of the early generations of inbreeding. Instead of the open pollinated seed, we used the O. P. + generation, representing one generation of sib pollination of the original open pollinated lines. For this sib pollination, the pollen of several tassels was mixed and applied to several ears and then the resultant seed was mixed. This was an attempt to increase the supply of seed of the open pollinated lines with the least possible inbreeding. Instead of the A generation of inbreeding, the b generation, representing two generations of sib pollination using a single plant each for pollen and ear, was substituted. Although this does not represent exactly the same amount of inbreeding as the A or A+ generation, it was the closest we could approximate it with the seed available and, consequently, was used. Comparison of the 1940 and 1941 (Figure 12) data will show a striking similarity between the two years and proves that we were justified in using the b instead of the A generation in produc-



ing crossed seed for the 1941 trials. Instead of the B and C generation, the B+ and C+ lines were used in the 1940 Purdue 39 crossing plot to produce seed for the 1941 trials. It is evident that inbreds making up the hybrids grown in 1941 represent a slightly more advanced stage of inbreeding than comparable 1940 crosses.

The hybrids in 1939 were not planted in a manner suitable for exact analysis and so the results reported here are based on the 1940 and 1941 trials.

In testing the combining ability of these inbreds crossed with a single inbred, Purdue 39, we are testing specific rather than general combining ability. In this experiment, it is not possible to separate the two as was attempted by Sprague and Tatum (1942). Further crosses with other inbreds would be necessary to do this. However, in sweet corn breeding, specific combining ability is probably equally as important as general combining ability, since all inbreds are utilized in single crosses and it is not necessary to know what yield factors an inbred will contribute to a double cross. Sweet corn breeding from now on will probably consist of finding new inbreds to give better hybrid combinations with already proven inbreds. The best way to determine this is to cross with the specific inbred itself. This was the procedure followed in this experiment and the results given are based on the combining ability of the several Whipple inbreds in different generations of inbreeding when crossed by Purdue 39.

## EFFECT OF INBREEDING ON INBREDS

Before analyzing the data of the hybrids, it will be well to examine the effect of inbreeding upon the inbreds themselves. The four generations of the different inbreds were grown in 1938 on a fairly uniform piece of soil. Measurements were made on a number of characters. These data are presented in Table 1.

### Effect of Inbreeding on Maturity

Figure 2 shows the date of tasseling and silking for each generation of inbreeding from none to three. As is customary in most inbreeding experiments, the time from planting to tasseling and silking lengthens slightly with the increased inbreeding. On the whole, the lines are parallel, showing a similar effect on tasseling and silking. This indicates that either days to tasseling or days to silking may be used as a satisfactory index of comparative maturity. In all lines, the date of tasseling is earlier than the silking date. The difference between the two varies with the different inbreds. For inbred 5, there is only a two day spread between average tasseling and silking while inbred 44 shows a difference of a week. For inbred 850-40 (now C23, pollen parent of Lincoln) and inbred 850-17 (now C27, pollen parent of Lee), the spread is four and five days, respectively. Such a spread is more satisfactory for inbreds used commercially than a spread of as

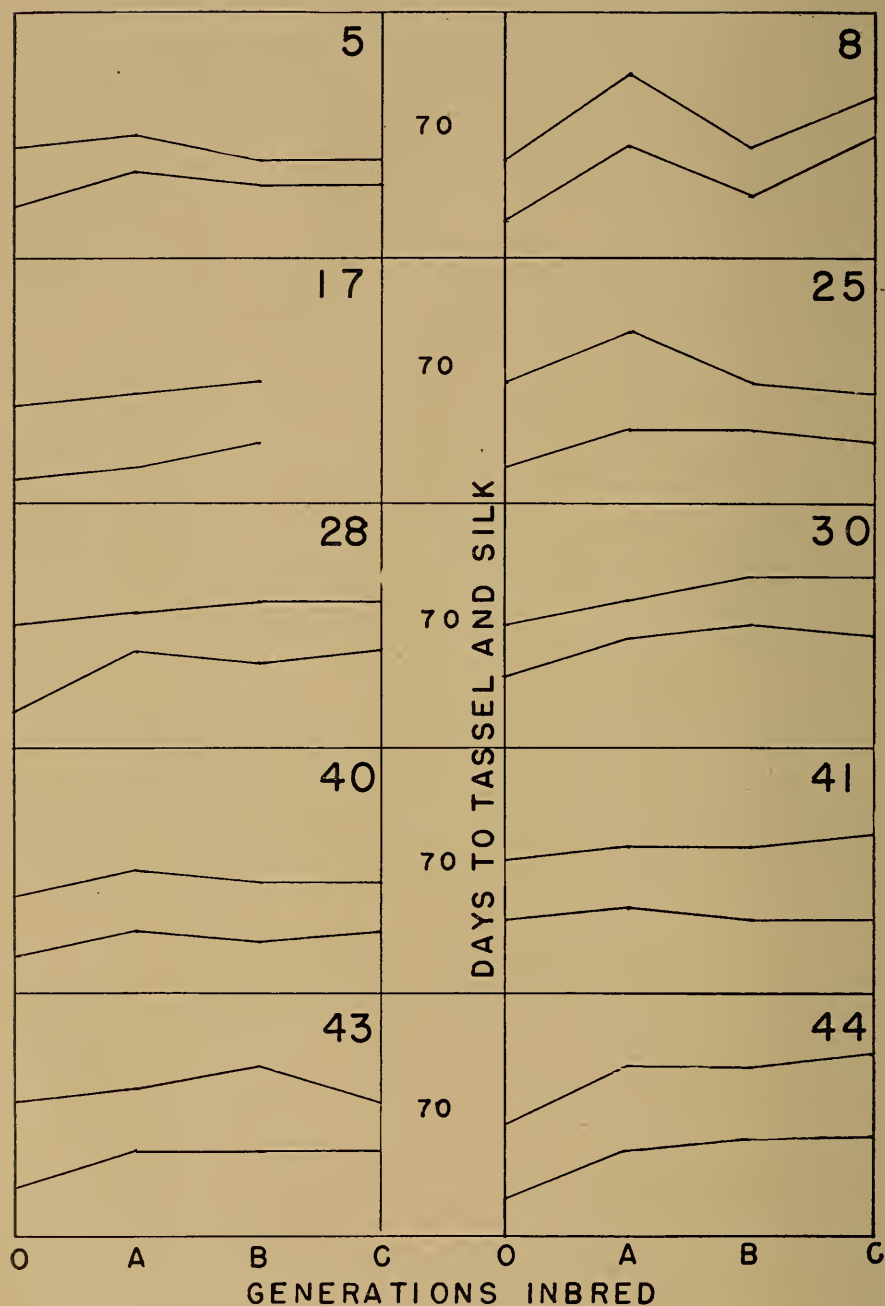


FIGURE 2. Days from planting to tasseling (lower line) and silking for 10 Whipple inbreds in four generations of inbreeding, grown in 1938.

TABLE I. AVERAGE NUMBER OF DAYS FROM PLANTING TO POLLEN SHEDDING AND SILKING WITH STANDARD DEVIATION (S. D.) FOR EACH: MEAN HEIGHT, WITH S. D. AND AVERAGE NUMBER OF TILLERS FOR TEN WHIPPLE INBREDS IN DIFFERENT STAGES OF INBREEDING.

Pedigree	Number of Plants	Days to Pollen	S. D.	Days to Silk	S. D.	Mean Height	S. D.	Mean Tillers
850-5 O. P.	145	64	3.0	69	3.2	77	11.7	1.34
" A	129	67	2.9	70	3.3	62	7.4	.86
" B	163	66	2.3	68	2.1	54	8.0	.02
" C	157	66	2.6	68	2.2	59	5.9	.00
850-8 O. P.	155	63	2.4	68	3.1	77	12.1	1.30
" A	.79	69	3.8	75	4.2	60	12.4	.06
" B	110	65	3.2	69	2.3	68	11.4	.41
" C	102	70	2.3	73	3.3	67	11.8	.22
850-17 O. P.	162	62	2.3	68	3.1	74	10.3	1.20
" A	142	63	2.2	69	3.9	63	7.6	.28
" B = C27	142	65	2.3	70	3.2	60	8.5	.02
850-25 O. P.	167	63	2.4	70	4.6	76	9.4	1.20
" A	97	66	2.5	74	4.4	61	8.1	.73
" B	112	66	3.2	70	2.9	52	6.6	.19
" C	153	65	3.7	69	3.0	59	4.8	.25
850-28 O. P.	139	63	2.8	70	3.6	77	11.1	1.00
" A	160	68	3.0	71	2.1	67	9.1	.15
" B	170	67	3.8	72	3.2	62	7.5	.06
" C	150	68	3.1	72	2.8	70	9.1	.03
850-30 O. P.	134	66	2.8	70	3.1	77	11.2	1.20
" A	138	69	3.0	72	3.2	66	10.5	.33
" B	93	70	3.8	74	4.1	64	10.1	.08
" C	48	69	2.6	74	4.0	72	9.6	.17
850-40 O. P.	138	63	2.4	68	2.7	75	10.2	.75
" A	148	65	3.9	70	3.5	71	7.8	.00
" B	106	64	3.0	69	1.9	69	6.9	.00
" C = C23	158	65	4.0	69	2.3	72	8.8	.00
850-41 O. P.	161	66	2.6	71	3.7	79	10.8	.40
" A	104	67	3.9	72	3.4	65	8.3	.10
" B	81	66	2.9	72	2.7	72	8.5	.00
" C	62	66	3.1	73	3.2	76	6.2	.16
850-43 O. P.	159	64	1.8	71	4.6	75	9.5	.42
" A	162	67	2.5	72	2.1	59	10.0	.17
" B	161	67	2.8	74	4.3	58	6.8	.00
" C	135	67	3.3	71	3.9	64	3.7	.04
850-44 O. P.	153	63	3.0	69	2.7	71	8.4	.54
" A	125	67	3.2	74	3.3	61	8.0	.82
" B	21	68	3.9	74	4.1	56	6.2	.05
" C	72	68	3.6	75	2.8	57	7.4	.70

much as a week, since such inbreds are sometimes difficult to self-pollinate.

After three generations of inbreeding, the different inbreds are about as variable in date of tasseling and silking as in the open pol-

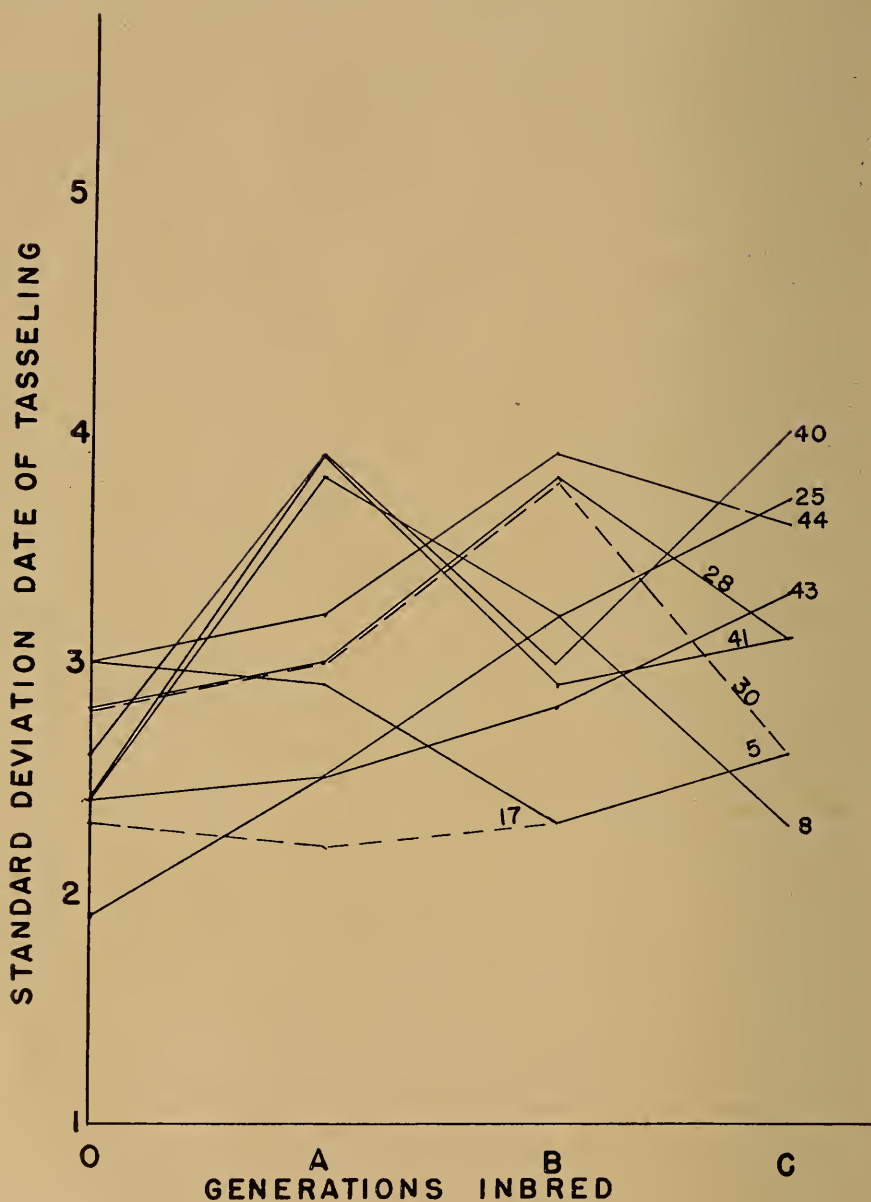


FIGURE 3. Standard deviation for date of tasseling of four different inbred generations of 10 Whipple lines grown in 1938.



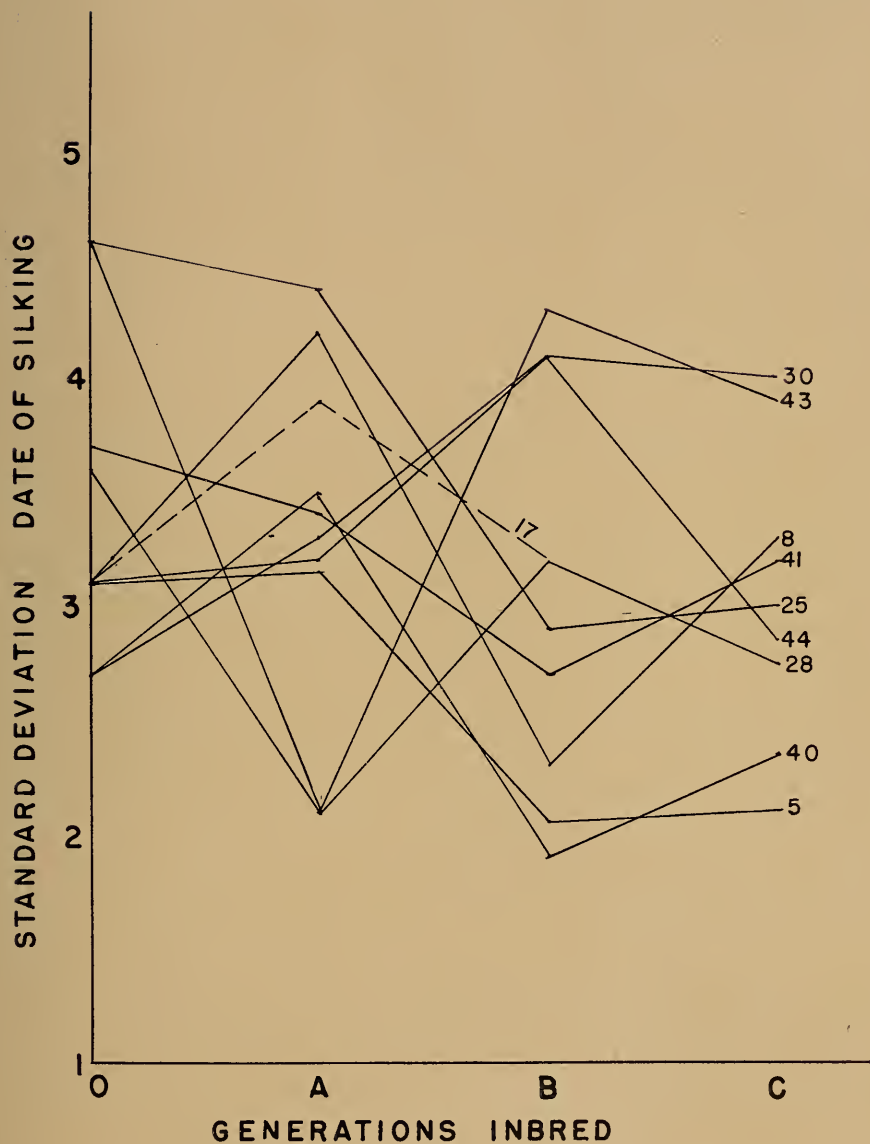


FIGURE 4. Standard deviation for date of silking of four different inbred generations of 10 Whipple lines grown in 1938.

linated original lines. This is shown by Figures 3 and 4 where standard deviation for tasseling and silking is plotted against generations of inbreeding. This would indicate that, after three generations of inbreeding, the lines are still segregating for maturity.

### Effect of Inbreeding on Height

One of the most pronounced effects of inbreeding in maize is a rapid reduction in height of the plants in the first few generations of inbreeding. After five or six generations of inbreeding, the inbreds have reached a comparatively stable position for height and change very slowly after that. Most of the decrease in height comes in the first three or four generations. Without selection, the plants of each succeeding generation are shorter than the previous one until a constant condition is reached.

Height of the ten Whipple inbreds in the different generations of inbreeding is plotted in Figure 5. It is evident from this chart that the height reduction behaves normally for the first two generations of inbreeding. In the third generation, however, there is a marked rise over the second for nearly all of the inbred lines. This is probably the effect of selection. In the early generations of inbreeding, the plants were being brought to a homozygous condition and the effect of inbreeding was operating to produce shorter plants in each succeeding generation. In the third generation, this downward trend was overcome, apparently by the effects of selection. We were not consciously selecting the tallest plants during the inbreeding process. However, we were selfing only one plant in each progeny. Consequently, we had to choose rather carefully the plant selfed in order to have some assurance of maintaining the line. Vigorous, healthy plants were chosen for selfing. Such selection, we believe, was primarily responsible for the upward swing in the height of the plants in the third generation of selfing.

In conjunction with the study of height of plants, we also compared the standard deviations for height in the different generations of inbreeding. As plants are inbred, we would expect them to become more uniform. If this is the case, the S. D. for each succeeding generation of inbreeding should be reduced. On the whole, this is what occurred as is seen in Figure 6. There are a few exceptions where the S. D. for the third generation was higher than for the second. This is not altogether surprising since nearly all of the inbreds increased in height, showing a possible selection of more heterozygous individuals as well as taller plants in the second generation.

### Effect of Inbreeding on Tillers

Tillering is more or less associated with vegetative vigor in sweet corn and is prevalent in most commercial open pollinated varieties. The Whipple variety produces tillers rather freely. As vegetative vigor is reduced by inbreeding, the tillering is also less profuse. Aside

from this association with vegetative vigor, there are also heritable differences between different lines for tillering. Figure 7 shows a graph for the tillering of ten inbreds in different stages of inbreeding. In all cases, the trend is downward. As the lines are reduced in vigor, the number of tillers also decreases. There are marked differences among the various lines for tillering, however.

The non-tillering habit became fixed very early in the case of 850-40. The first generation selfed was free from tillers and the line has remained so ever since. This line is now C23, the pollen parent of Lincoln. C23 has comparatively few tillers and imparts this character to Lincoln.

The inbred 850-5 was not fixed for non-tillering until after the second selfed generation. A study of Figure 7 will reveal other obvious differences in the tillering habit of the various inbred lines.

## **EFFECT OF INBREEDING ON COMBINING ABILITY**

While the effect of inbreeding upon the inbreds themselves is interesting, the thing with which we are primarily concerned is the effect of inbreeding on combining ability. The following sections are concerned with the effect of inbreeding upon the hybrids resulting from crossing by Purdue 39 the Whipple lines in different stages of inbreeding.

### **Effect of Inbreeding on Yield of Hybrids**

In 1940 and 1941, the ten Whipple lines, each with the open pollinated ear and three generations of inbreeding crossed by P39, were put into split plot tests. In each year, the test consisted of 200 individual plots divided into five blocks. Each block was divided into ten sub-blocks, each sub-block containing one line. Within the sub-block of each line, there were four randomized plots, representing the different degrees of inbreeding for that line.

At harvest time, only one ear was taken from each stalk since most of the hybrids were single-eared and only nubbins, if any, were produced as second ears. The ears were then dried in the seed barn to constant weight before being weighed. The weight was taken in grams and, for ease in calculation, row totals were divided by ten.

When the data for yield of ear corn in the years 1940 and 1941 were examined, discrepancies in stand were found to have affected total yields so markedly that an initial analysis of variance showed no relation between yield and generation of inbreeding.

The usual assumption that plants in incomplete rows tend to adjust yield inequalities by an increased production per plant seems to be invalid here since differences in yield between varieties, between

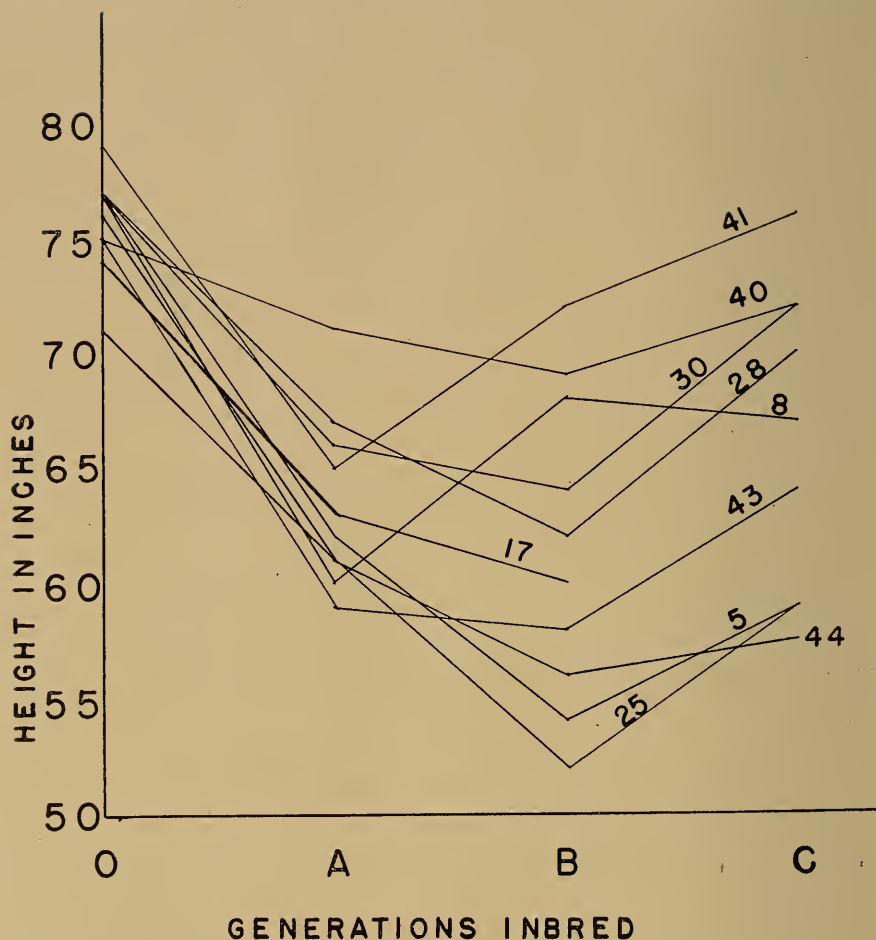


FIGURE 5. Height of four inbred generations of 10 Whipple lines grown in 1938.

generations of inbreeding, or between blocks were wholly dependent on the number of plants within the category. Such a situation required correction for stand. Dividing yield by the number of plants to find the production per plant is generally considered an arbitrary and unjustified correction (Kiesselbach, 1923), since it assumes that every plant in a row, whether the first or fifteenth, will add the same amount to the row total. This would happen only when each plant was given so much area as to eliminate competition between plants.

An alternative correction is to derive a regression coefficient relating yield to the number of plants within categories by the analysis of covariance and to correct difference in stand with this observed relation. Efficient use of this type of correction depends on two

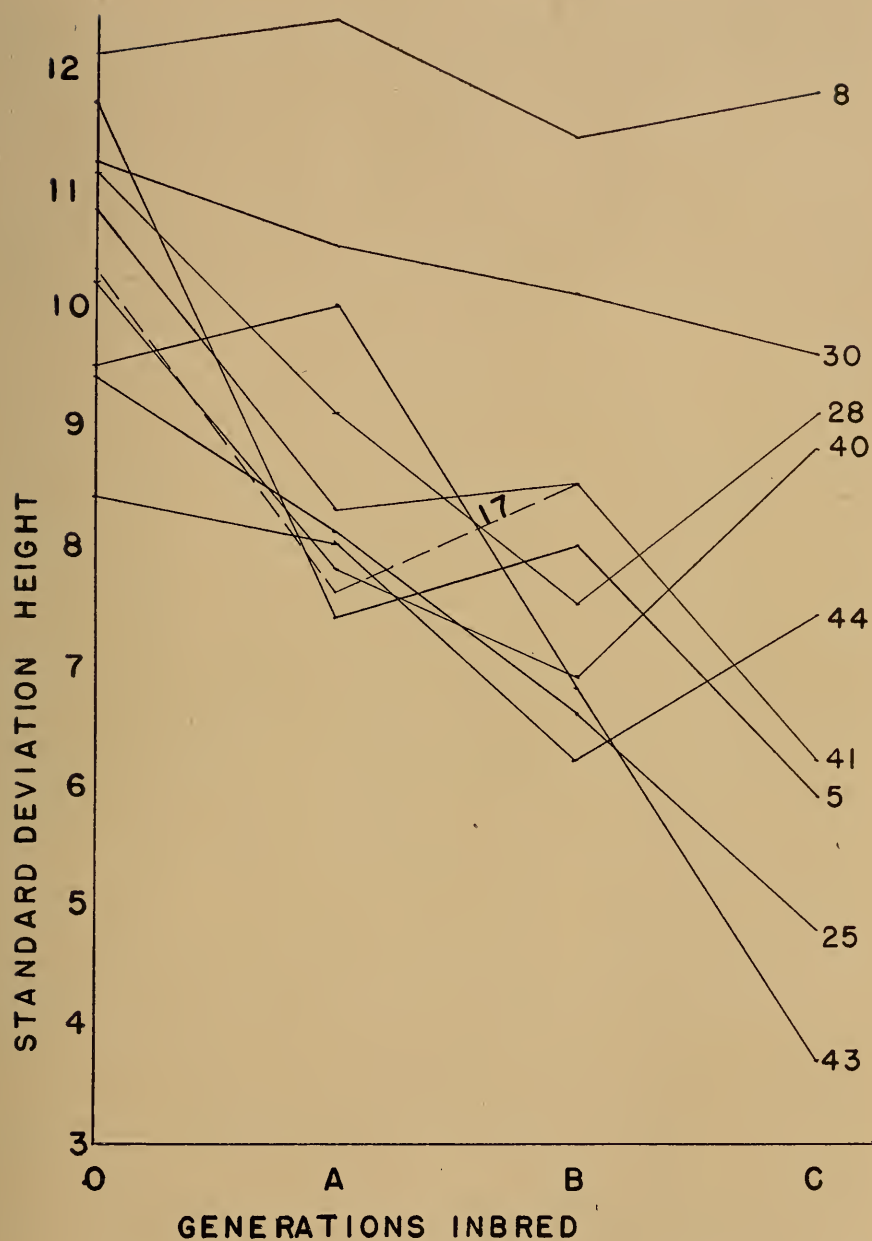


FIGURE 6. Standard deviation for height of plants of four inbred generations of 10 Whipple lines grown in 1938.

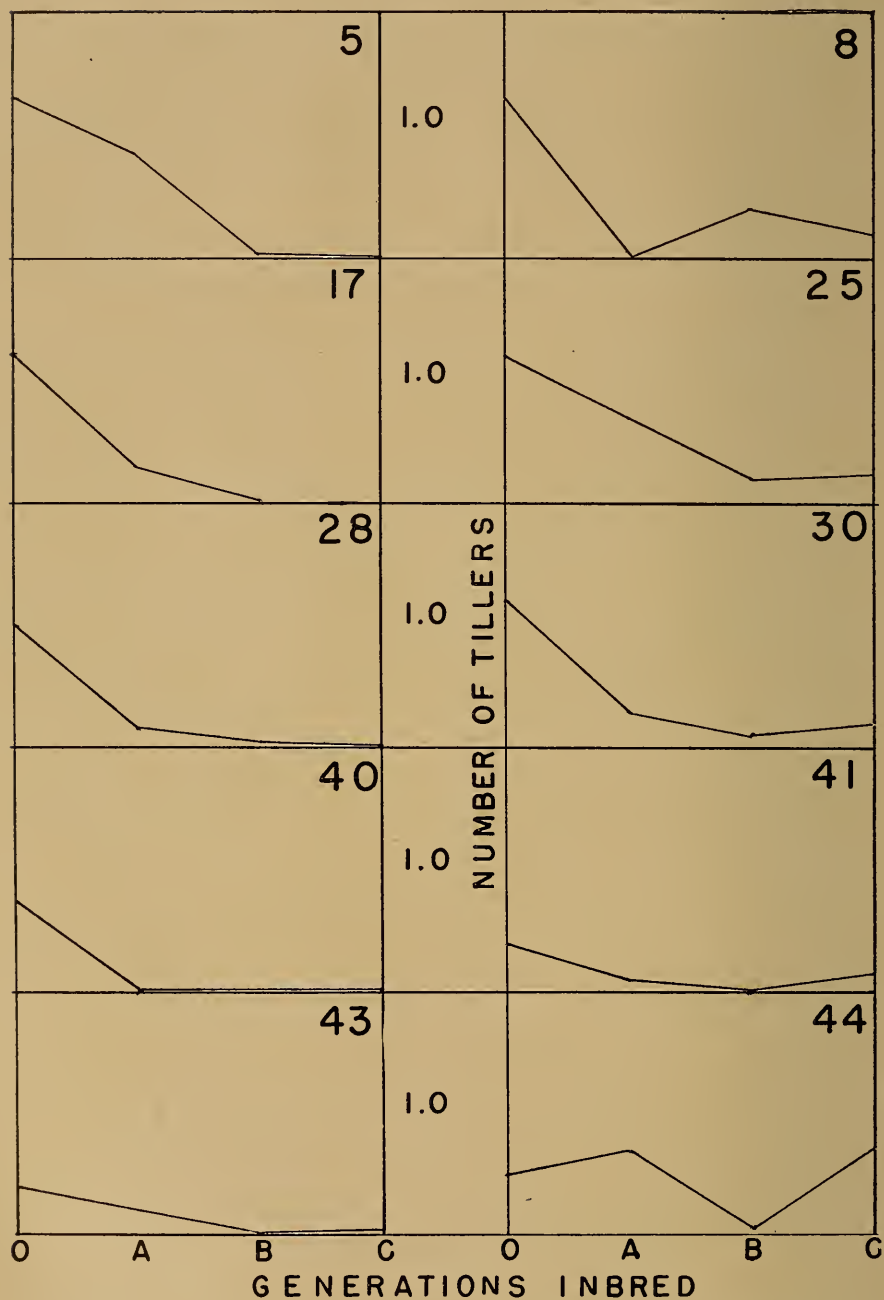


FIGURE 7. Average number of tillers per plant for 10 Whipple lines in four different generations of inbreeding grown in 1938.



factors. (1) Within the range where correction is needed, there must be a linear relationship between yield and the number of plants. (2) The regression coefficient within individual lines and generations of inbreeding should form a homogeneous series which can be pooled, so that stand can be corrected by a single term.

The first of these requirements is examined in Figures 8A and B, where yield is plotted against the number of plants for varieties and blocks, respectively, in both 1940 and 1941. In both figures, there is good agreement with the assumption of linear relationship in this range between yield and number of plants. Table II gives the regression coefficient and the number of plants for each generation of inbreeding within each line for 1940 and Table III those for 1941.

TABLE II. REGRESSION COEFFICIENTS. 1940

Varieties	0 Gen. Inbred b No. Plants		1st. Gen. Inbred b No. Plants		2nd. Gen. Inbred b No. Plants		3rd. Gen. Inbred b No. Plants	
850-5	11.401	104	11.979	81	11.612	102	10.472	91
40	10.904	79	10.851	86	11.858	91	11.926	99
8	11.170	75	13.213	52	12.766	65	12.371	85
25	11.731	78	11.890	66	11.532	86	11.030	70
43	11.705	82	10.508	73	11.727	75	12.333	73
17	11.852	79	10.950	78	10.401	89	10.100	83
44	10.695	82	11.984	75	11.727	88	12.896	77
28	11.866	90	11.476	76	11.562	89	12.617	79
30	11.102	87	11.682	72	11.524	81	11.682	63
41	10.975	77	11.322	85	11.462	75	12.809	84

TABLE III. REGRESSION COEFFICIENTS. 1941

Varieties	0 Gen. Inbred b No. Plants		1st. Gen. Inbred b No. Plants		2nd. Gen. Inbred b No. Plants		3rd. Gen. Inbred b No. Plants	
850-5	13.54	97	12.99	93	13.63	95	12.11	91
40	13.14	93	12.70	103	13.66	103	14.32	100
8	12.70	87	12.65	91	13.76	89	14.98	79
25	13.54	77	13.50	92	13.84	81	13.31	85
43	13.87	88	12.44	91	13.28	90	13.92	85
17	13.87	90	13.26	69	14.49	91	14.20	94
44	13.47	88	13.53	90	12.84	86	13.37	83
28	13.49	89	13.20	85	12.59	97	13.86	90
30	13.06	98	12.86	91	13.35	87	13.46	72
41	12.55	98	12.54	83	13.12	92	14.48	89

Tables IV and V give the collected regression coefficients for each generation (all lines) and each line (all generations), respectively, compared to number of plants for each case.

TABLE IV. REGRESSION COEFFICIENTS BY GENERATION OF INBREEDING

Generation of Inbreeding	1940		1941	
	b	No. Plants	b	No. Plants
0	11.35	833	13.30	905
1	11.47	744	12.95	888
2	11.49	841	13.46	911
3	11.81	804	13.81	868

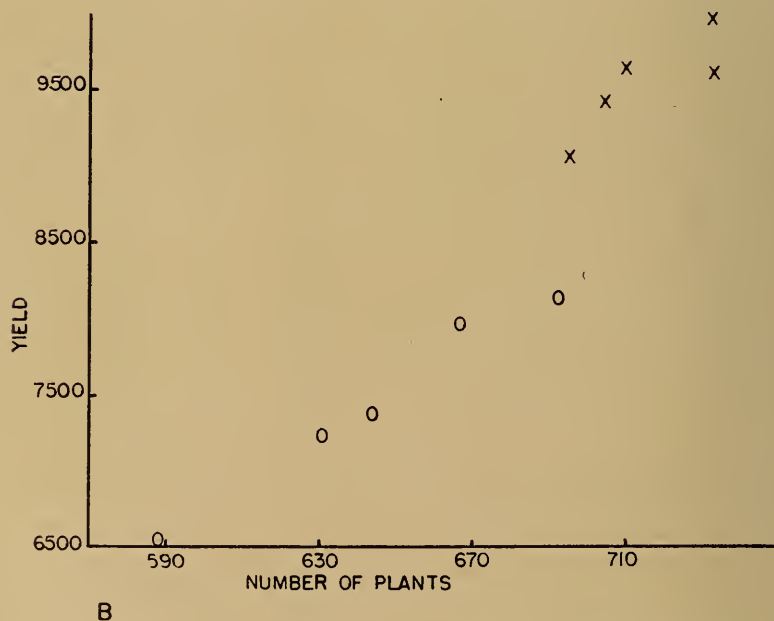
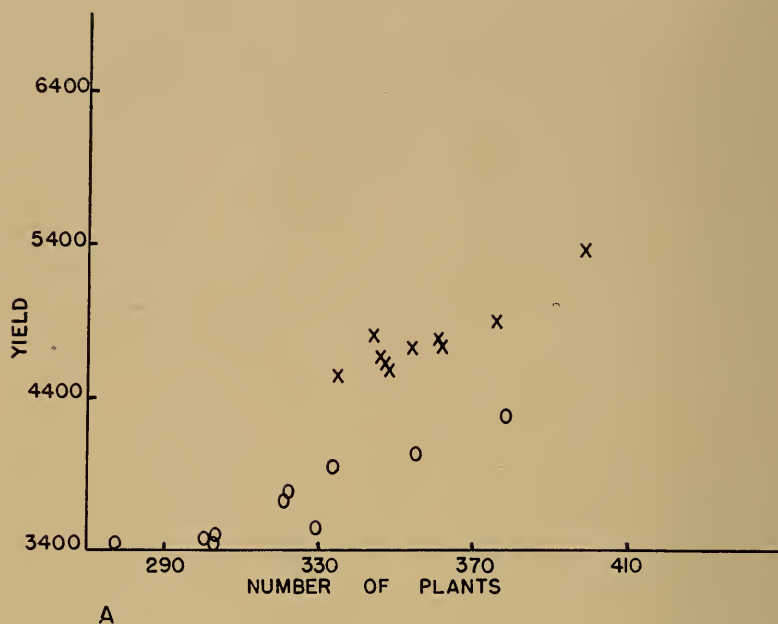


FIGURE 8A. Unadjusted yields in grams by number of plants for each variety. The circles represent 1940 and the crosses, 1941.

FIGURE 8B. Unadjusted yields in grams by number of plants for each block. The circles represent 1940 and the crosses, 1941.

TABLE V. REGRESSION COEFFICIENTS BY VARIETIES

Varieties	1940		1941	
	b	No. Plants	b	No. Plants
850-5	11.36	378	13.11	376
40	11.48	355	13.46	399
8	12.22	277	13.44	346
25	11.55	300	13.54	335
43	11.57	303	13.47	354
17	10.77	329	14.03	344
44	11.79	322	13.33	347
28	11.87	334	13.26	361
30	11.45	303	13.15	348
41	11.68	321	13.15	362

None of these tables shows a correlation between plant number and the regression coefficients which would invalidate correction by a linear regression term. Nor are there large differences between the regression coefficients for different generations of inbreeding or different varieties except for the first inbred generation in 1941 which is lower than expected. The analysis of these regression coefficients is shown in Table VI.



FIGURE 9. Ears of Purdue 39 (left), Lee and C27 (850-17). Note that C27 is almost as large as the hybrid. C27 is earlier than Lee or P39. Note wrinkling of kernels.

TABLE VI. ANALYSIS OF REGRESSION

Source	DF	1940		1941	
		MS	F	MS	F
Effect of Pooled Regression	1	7165823	3646.73**	11629536	69223.43**
Between Varieties	9	724	.37	474	2.82
Between Generations	3	646	.33	2009	11.96**
Generation $\times$ Varieties	27	1965		168	

In the 1940 test, the small F values between varieties and between generations and the large F value for the effect of pooled regression justify the use of a single pooled regression coefficient for correcting stand. In view of the significant F value between generations in the 1941 test, adjustment with a combined b introduces a small but consistent bias. This bias affects the yield values for the first inbred generation which is the only generation significantly different from the rest. However, the over-all picture is not changed by using a separate b for this generation. In general, there was good agreement within varieties between the two years and the mean square for generations by varieties in 1941 was less than 10 per cent as large as in 1940, which exaggerated the contrast between generations. For these reasons, the 1941 data have been corrected for stand on the same basis as the 1940 test.

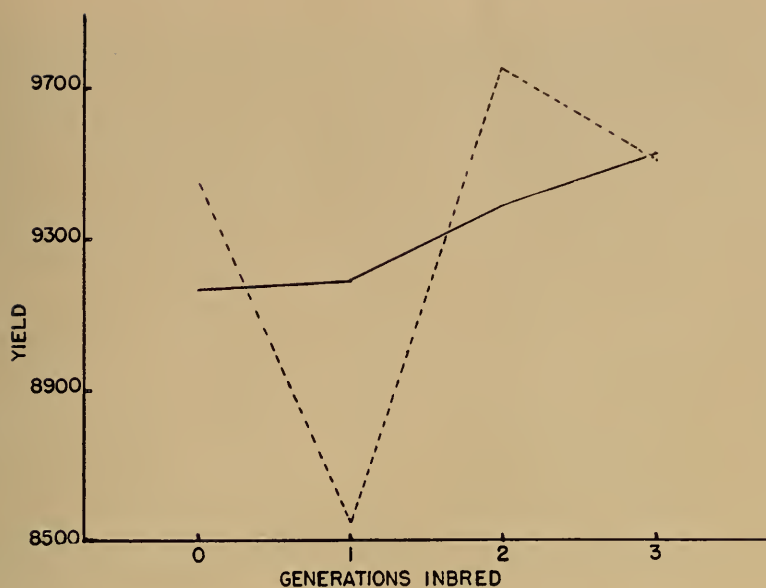
The combined regression coefficient b for the correcting of inequalities in stand over the whole test is derived from an analysis of covariance where w = number of plants and y = plot yield. The calculation parallels the analysis of variance for the same test but with the addition of  $w^2$  and the product wy for every  $y^2$ . The pooled regression coefficient is derived from the ratio of the product wy to  $w^2$  in the error row of the table. Plot yields are then corrected to a con-

TABLE VII. ANALYSIS OF COVARIANCE. 1940

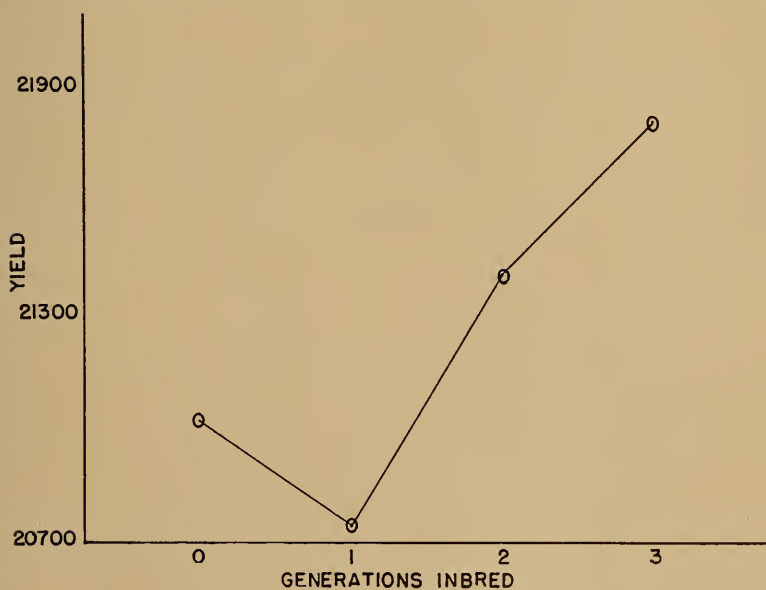
w = No. of Plants  
y = Plot Yield

Source of variation	DF	SS ( $w^2$ )	SS (wy)	SS ( $y^2$ )	SS Adj. ( $y^2$ )	MS Adj. ( $y^2$ )	F
Blocks	4	153.43	2454.74	40165.47	5986.50	1496.63	4.21**
Varieties	9	382.48	3653.91	39595.15	4867.44	540.83	1.52
Plot Error	36	239.17	3189.71	53022.13	12780.56	355.02	
Gen. Inbreeding	3	116.02	1309.97	16409.30	1747.53	582.51	3.61*
Linear	1	.10	13.75	1890.63	1619.62	1619.62	10.03**
Quad.	1	13.52	168.22	2093.05	65.78	65.78	.41
Cubic	1	102.40	1128.00	12425.63	62.16	62.16	.39
Gen. $\times$ Var.	27	318.28	3531.33	53824.15	14878.47	551.05	3.41*
Error Term	119	612.20	6266.95	83376.80	19223.69	161.54	
Total	198	1821.58	20406.61	286393.00	59484.19		
C. F.	1	51906.42	600081.39	6937440.00			

$$b = 6266.95/612.20 = 10.236769$$



A



B

FIGURE 10A. Yield in grams by generation of inbreeding for 1940. The broken line represents unadjusted yields. The solid line represents adjusted yields.

FIGURE 10B. Adjusted yield in grams by generation of inbreeding combining 1940 and 1941 results.



stant stand by the equation  $y' = y + b(\bar{w} - w)$ . The sum of squares of the adjusted  $y^2$  can be determined for each term in the analysis as  $b^2 (w^2) - 2b(wy) + y^2$ , without correcting each plot individually and recalculating the analysis of variance. Table VII shows the complete analysis of covariance for the year 1940.

Note that the row for error combines the values for blocks  $\times$  generation and varieties  $\times$  blocks  $\times$  generation, since they are of about the same magnitude and the interaction of blocks  $\times$  generation is of little intrinsic interest.

Table VII shows the large increase in precision obtained by correcting discrepancies in stand by the analysis of covariance. The mean square for the error of a single plot with 119 degrees of freedom is reduced from 700.65 in unadjusted ( $y^2$ ) to 161.54 in the adjusted ( $y^2$ ). Further, large and inexplicable differences in the yield of different generations and varieties were apparently due to unequal numbers of plants, since correction for stand brings about a more nearly expected relationship (Figure 10 A).

Table VII gives only the sums of squares for the adjusted ( $y^2$ ) instead of the reduced ( $y^2$ ) which would be used for a critical test of significance for a single season. This table illustrates the method but the conclusions are drawn from a combined analysis of the adjusted values for the two years 1940 and 1941 (Table VIII). For this purpose, the data for 1941 were treated similarly.

TABLE VIII. ANALYSIS OF VARIANCE FOR YIELD. 1940 and 1941

Source of Variation	DF	MS	F
Blocks	8	1194.81	2.91*
Years	1	275362.56	66.99**
Varieties	9	230.49	.56
Varieties $\times$ Years	9	722.66	1.76
Var. $\times$ Blks. $\times$ Yrs.	72	411.08	
Generations	3	2163.57	14.35**
Linear	1	4586.95	30.42**
Quad.	1	1170.96	7.75*
Cubic	1	732.79	4.86
Generations $\times$ Years	3	296.04	1.96
Generations $\times$ Var.	27	885.76	5.87**
Gen. $\times$ var. $\times$ Yrs.	27	212.88	1.41
Error Term	238	150.80	

Three terms in Table VIII are of particular importance, varieties, generation of inbreeding and varieties by generation. (1) Varieties have a low F value indicating that, over both years and considering all generations, no variety is significantly better than any other. Nor was the interaction of years on varieties significant. Although some varieties may have reacted differently in the two years, these differences were not large enough to approach significance.



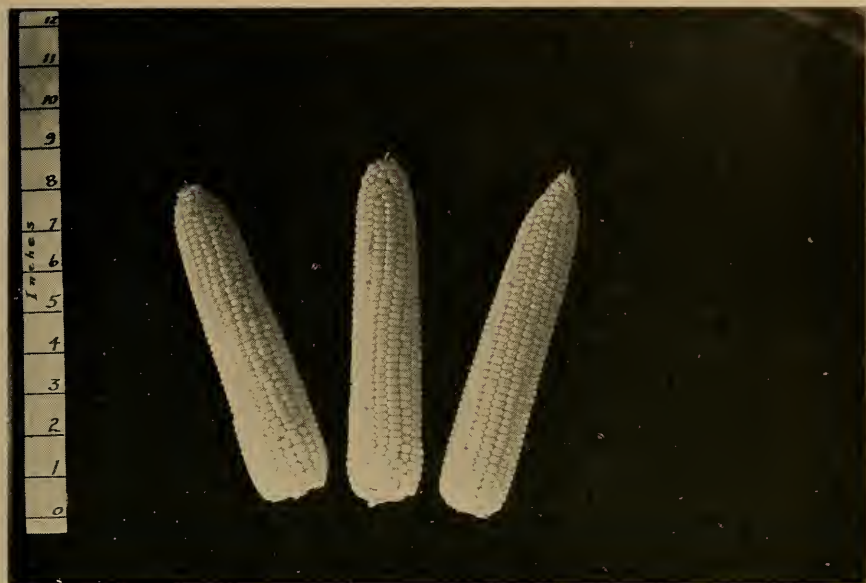


FIGURE 11. Lincoln sweet corn, P39  $\times$  C23 (850-40), a productive midseason hybrid produced during the course of the investigation reported here.

(2) The  $F$  value for generations of inbreeding is highly significant, so that at least two generations of inbreeding differed significantly. The group means are 210 for 0 generations of inbreeding, 207 for one generation, 214 for the second and 218 for the third. A difference of 4.5 is significant at the .01 level. Considering all varieties, the yield of the third inbred generation is significantly higher than the 0 generation. On the average, selection of plants deemed most promising for inbreeding has been effective in raising the mean yield in later generations.

The relation of yield to generation of inbreeding cannot be expressed by a linear relationship (Figure 10B). When the sum of squares for generation of inbreeding is divided into three components, (a) the linear term—that attributable to a straight line relating generation of inbreeding to yield, (b) systematic curvature from this line in one direction—the quadratic term, and (c) residual variance (the cubic), the first two terms proved significant but not the third. Hence, the relation between inbreeding and yield may be expressed by a line with a single curvature.

(3) Since the experiment was designed to compare the tendency of different varieties to maintain a level established in the 0 or the first generation of inbreeding, the term varieties  $\times$  generations is of interest. The  $F$  value indicates that this interaction was significant. Therefore, the varieties did not react alike to the same degree of in-

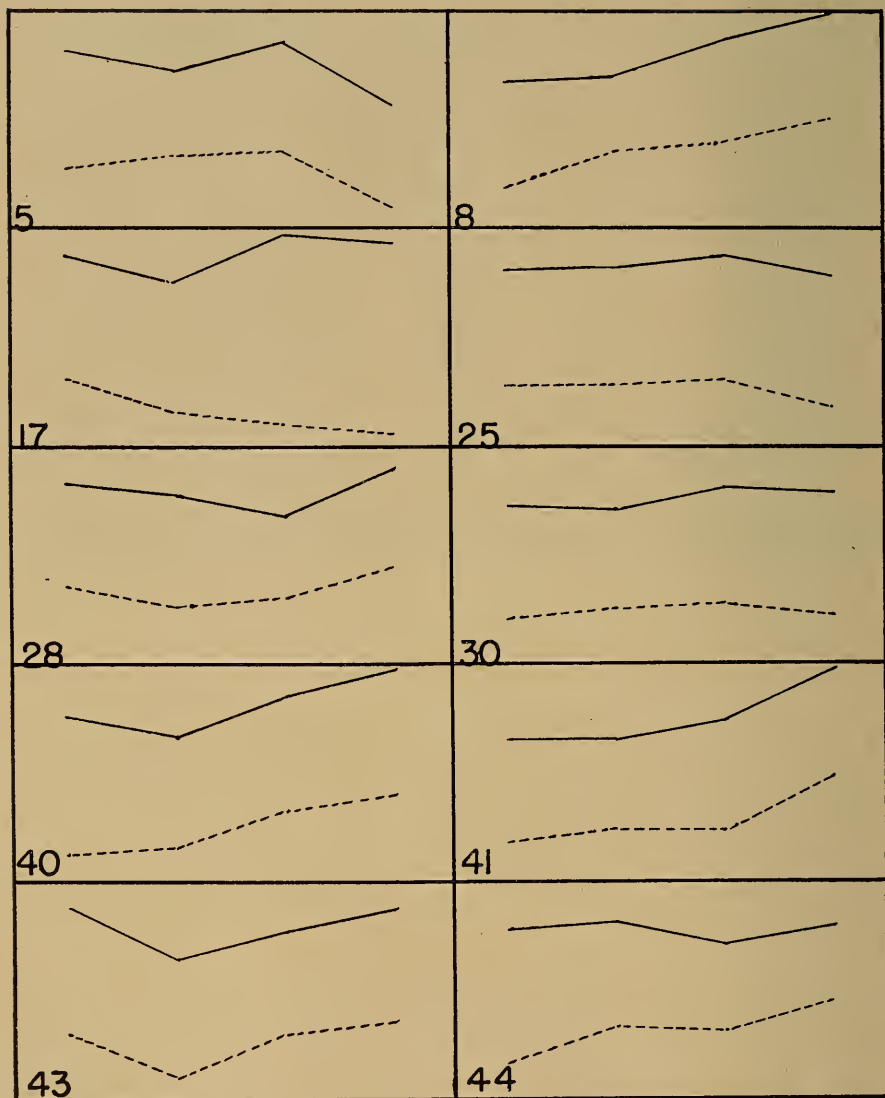


FIGURE 12. Adjusted yields in grams by generation of inbreeding for each variety. The broken line represents 1940 and the solid line, 1941.

breeding. This is shown clearly by Figure 12 where each variety yield is plotted against generation of inbreeding separately for the years 1940 and 1941. Varieties 25 and 30 are the only lines where the yield observed in the 0 generation was maintained through three generations of inbreeding. All other varieties increased or decreased appreciably from the 0 to the later generations. It is generally as-

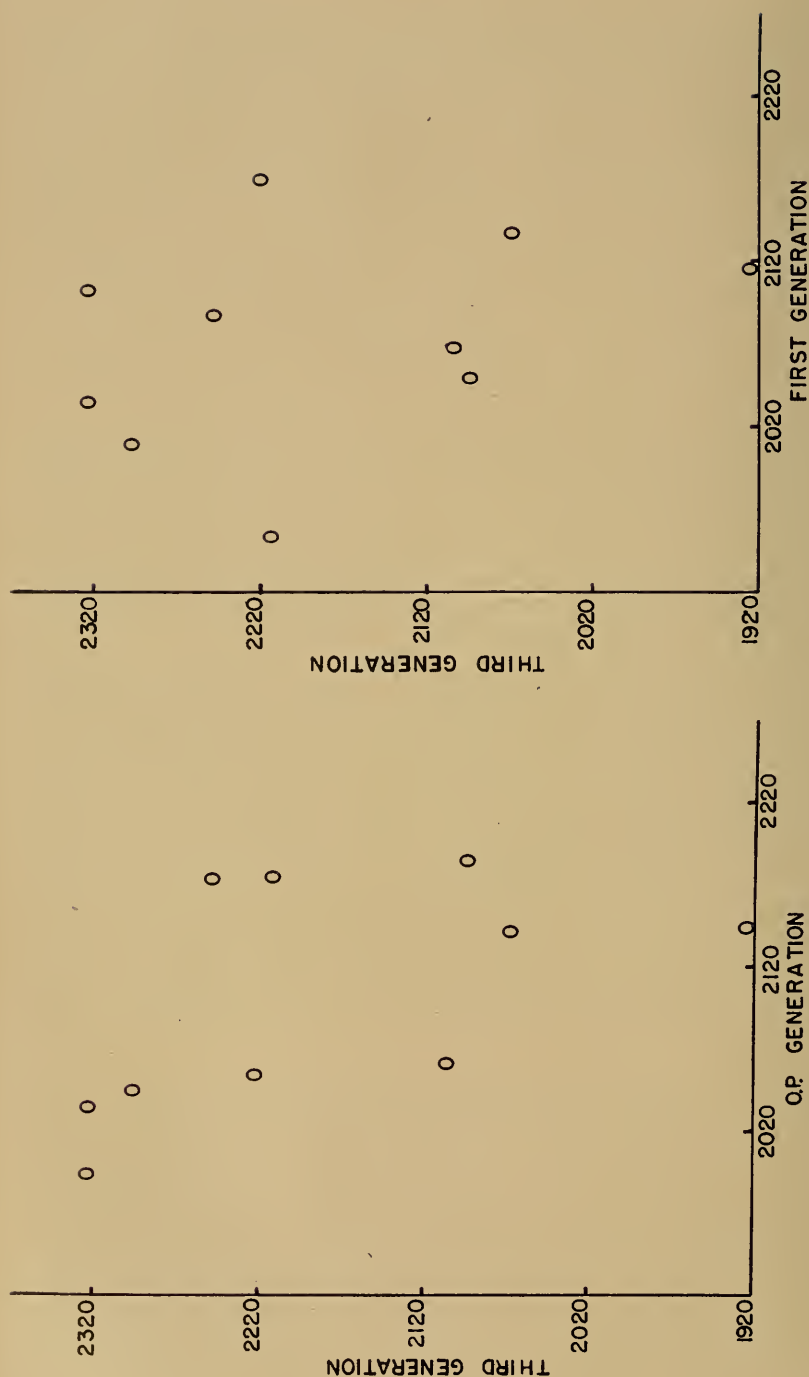
sumed that yield factors are sufficiently well fixed by the third generation of inbreeding that subsequent inbreeding will reduce heterozygosity but not alter materially the factors affecting yield. It was thought that a significant correlation might occur between yield in the third generation and the yield in a preceding generation. Were this possible, large scale testing in the early stages of inbreeding would enable the plant breeder to select his best lines early in the inbreeding process. However, in these ten Whipple lines, the yield in the third generation was not correlated with that in the second, first, or 0 generations, as is shown in Figures 13 A, B and C, where the third generation yield has been plotted against the 0, first and second generation yields, respectively. For example, the five varieties with the highest second generation yield, (Figure 13C) in the third generation classed as two high yielding and three low yielding varieties. Similar examples are present in Figures 13 A, B and D.

In the third generation, we are approaching the point where yield factors should be positively correlated with yield factors in subsequent generations. Since yield in 0, first or second generations showed little or no correlation with that in the third generation, these early generation yields can scarcely be expected to give valuable clues to the yield factors which will be present in these inbreds in the fourth, fifth, and sixth generations. Thus, testing of inbreds in the first or second generation of inbreeding seems to be of little use.

It is of interest to note the good agreement in yield in each variety between different years. The good growing conditions of 1941 as contrasted with the wet, cold summer of 1940 resulted in yields that were approximately 23 per cent higher in 1941. Yet, with one exception, the relative yields of all varieties agreed well in these two years. No. 850-17, on the other hand, responded quite differently to the different environmental conditions of 1940 and 1941. In 1940, of the ten varieties, 17 was the least affected by unevenness of stand with a regression coefficient of 10.77 while, in 1941, the relation was reversed and 17 had the highest regression coefficient of 14.03. This line has since been designated as C27 and is crossed by P39 to give the mid-season hybrid, Lee (Figures 9 and 14). The same tendency to produce well in good years and poorly in bad years still persists to a remarkable degree.

### **Mean Length of Ear in Relation to Inbreeding**

The relation of ear size to the generation of inbreeding after crossing by a common inbred was studied because of its obvious correlation to total yield and, also, because it was believed to be relatively independent of unevenness in stand so that no correction for stand would be necessary. It was shown in a preceding section that the process of inbreeding was effective in raising the average yield from generation to generation except for a non-significant drop from the 0 to the first inbred generation. This increasing yield is evidence of a favorable selection among the innumerable factors which influence

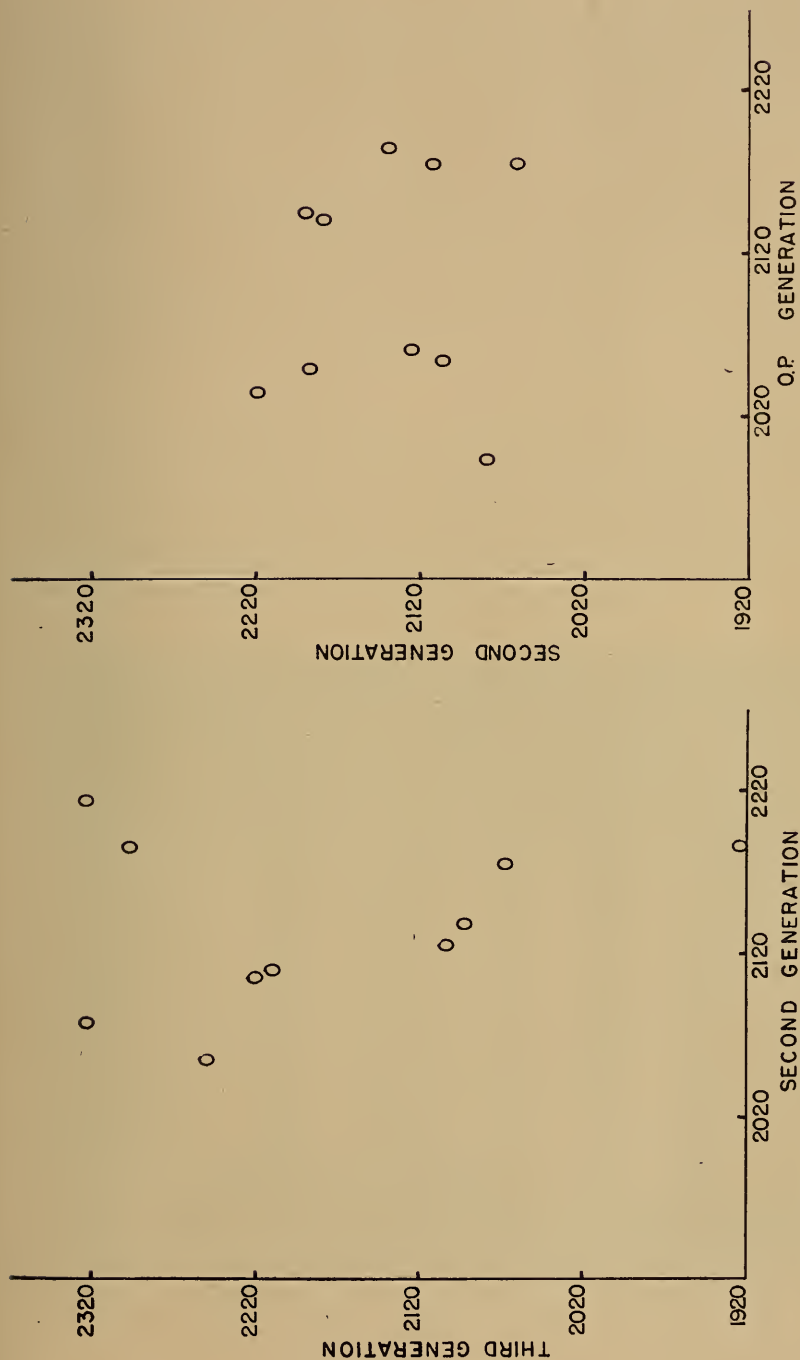


A

B

FIGURE 13A. Yield in the third inbred generation by yield in the open pollinated generation for each variety combining 1940 and 1941 results.

FIGURE 13B. Yield in the third inbred generation by yield in the first inbred generation for each variety combining 1940 and 1941 results.



C

D

FIGURE 13C. Yield in the third inbred generation by yield in the second inbred generation for each variety combining 1940 and 1941 results.  
FIGURE 13D. Yield in the second inbred generation by yield in the open pollinated generation for each variety combining 1940 and 1941 results.



yield to a greater or lesser degree. One of the more important of these factors is length of ear. The effect of length of ear on yield could have been segregated by covariance as with plant stand. This was not done since we were concerned mainly with the over-all effect of inbreeding on yield and not the separation of the factors contributing to this effect.

With emphasis during the inbreeding process on appearance of the plants at the time of pollination, and attractiveness of the ears at time of harvest rather than conscious breeding for length of ear, what is the effect of inbreeding on the length of ear? In the inbreeding process per se with the cumulative effects of homozygosity, mean length of ear decreases from generation to generation, but when all generations are crossed by a common inbred a very different relationship exists.

When mean length of ear in cm. is plotted against generation of inbreeding (Figure 15), the result is a straight line showing a gradual and steady increase in length from generation to generation. Table IX is a combined analysis of variance for the years 1940 and 1941. The variance ratio for generations shows that the four differed significantly. The averages for the four generations are 0, 160.5; 1, 162.8; 2, 165.0, and 3, 167.8, with a least significant difference on the .01 level of 3.7. Apparently, considering all varieties, the inbreeding

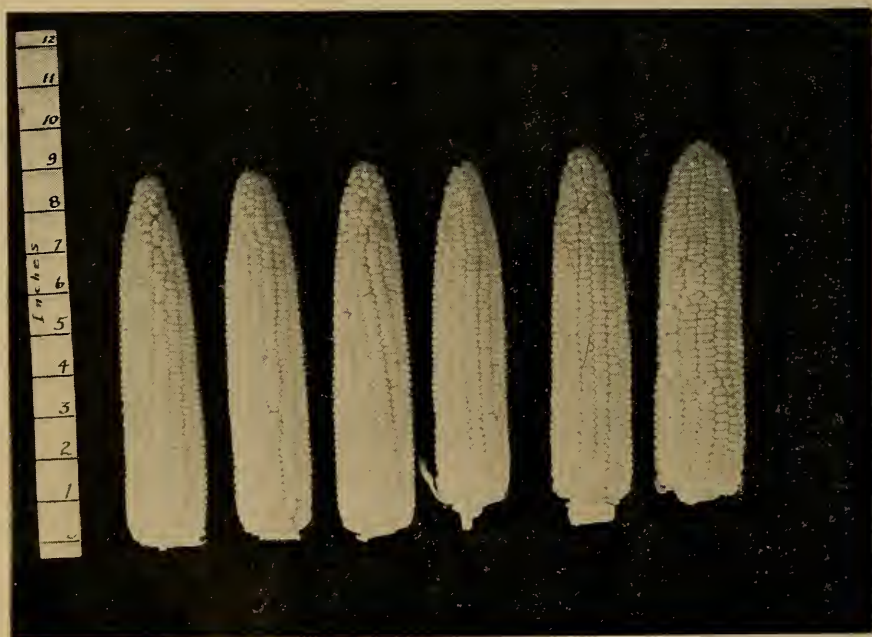


FIGURE 14. Six ears of Lee sweet corn, P39  $\times$  C27, another productive mid-season hybrid developed during the course of this investigation.



TABLE IX. ANALYSIS OF VARIANCE FOR MEAN LENGTH OF EAR. 1940 and 1941

Source of Variation	DF	MS	F
Blocks	8	205.30	3.38**
Years	1	10660.56	175.34**
Varieties	9	236.74	3.89**
Varieties $\times$ Years	9	179.55	2.95**
Var. $\times$ Blks. $\times$ Yrs.	72	60.80	
Generations	3	975.26	9.21**
Linear	1	2916.11	27.53**
Quadratic	1	7.56	
Cubic	1	2.11	
Gen. $\times$ Years	3	134.83	1.27
Gen. $\times$ Var.	27	228.53	2.16**
Gen. $\times$ Var. $\times$ Yrs.	27	105.00	1
Blks. $\times$ Gen. $\times$ Var. $\times$ Yrs.	238	105.93	

process increased ear length in the hybrid from generation to generation. The relation between degree of inbreeding and ear length is best expressed by a straight line as shown by the separation of the sum of squares for the term generation of inbreeding into linear, quadratic and cubic terms. The linear term is highly significant while the terms for curvature are negligible.

Despite the general increase for all varieties, the significance of the interaction between generation and variety shows that all of the varieties did not react alike to the generation of inbreeding. This is further demonstrated by Figure 16 where, for each of the ten varieties, ear length is plotted against generation for the two years 1940 and 1941. It is apparent that in some lines the general trend was up and in others down, with still others remaining almost unchanged. With the exception of varieties 17 and 8, there was good agreement between years.

### Standard Deviation Length of Ear

In addition to the mean length of ear, the standard deviation in the length of ear was also computed. Presumably, as the degree of inbreeding and, hence, the homozygosity of a stock increases, the standard deviation for any given character should decrease. What will happen when these stocks at different degrees of inbreeding are outcrossed to a common inbred? Two possibilities may be suggested. First, the hybrids of different degrees of inbreeding might reflect more or less closely the differences in standard deviation of the female parents. Secondly, the male parent in common to all these crosses might be homozygous for so many dominant genes affecting ear length that differences between the dissimilar female parents might be smoothed out.

In this case, as may be seen by reference to Figure 17 where standard deviation of ear length in the crosses is plotted against generation

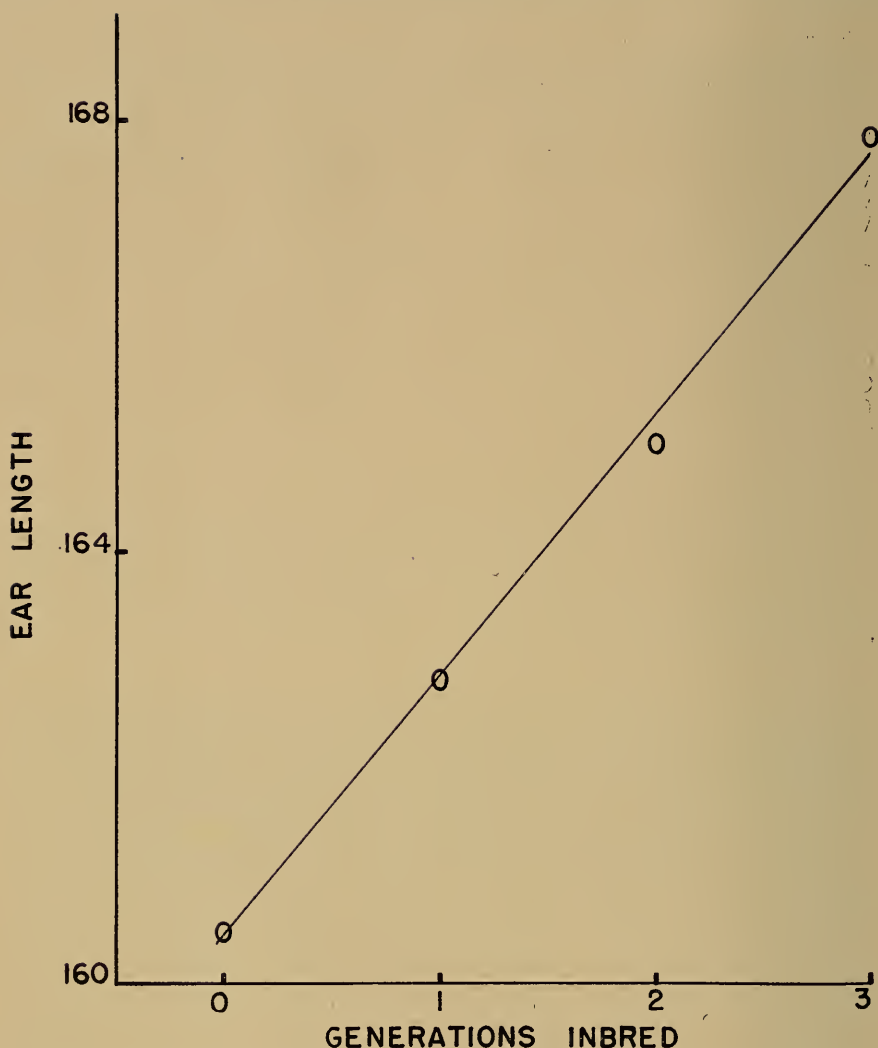


FIGURE 15. The mean length of ear in centimeters by generation when crossed by P39, for inbreeding for all varieties and combining 1940 and 1941 results.

of inbreeding of the female parents, the first condition would seem to apply. There is a slight drop from the 0 to the first inbred generation, a marked decrease from the first to the second generation, and a rise from the second to the third generation. Since differences between the second and third were not significant, the possibility remains that there may be a continuous downward trend.

The analysis of variance for standard deviation length of ear in the years 1940 and 1941 is shown in Table 10.

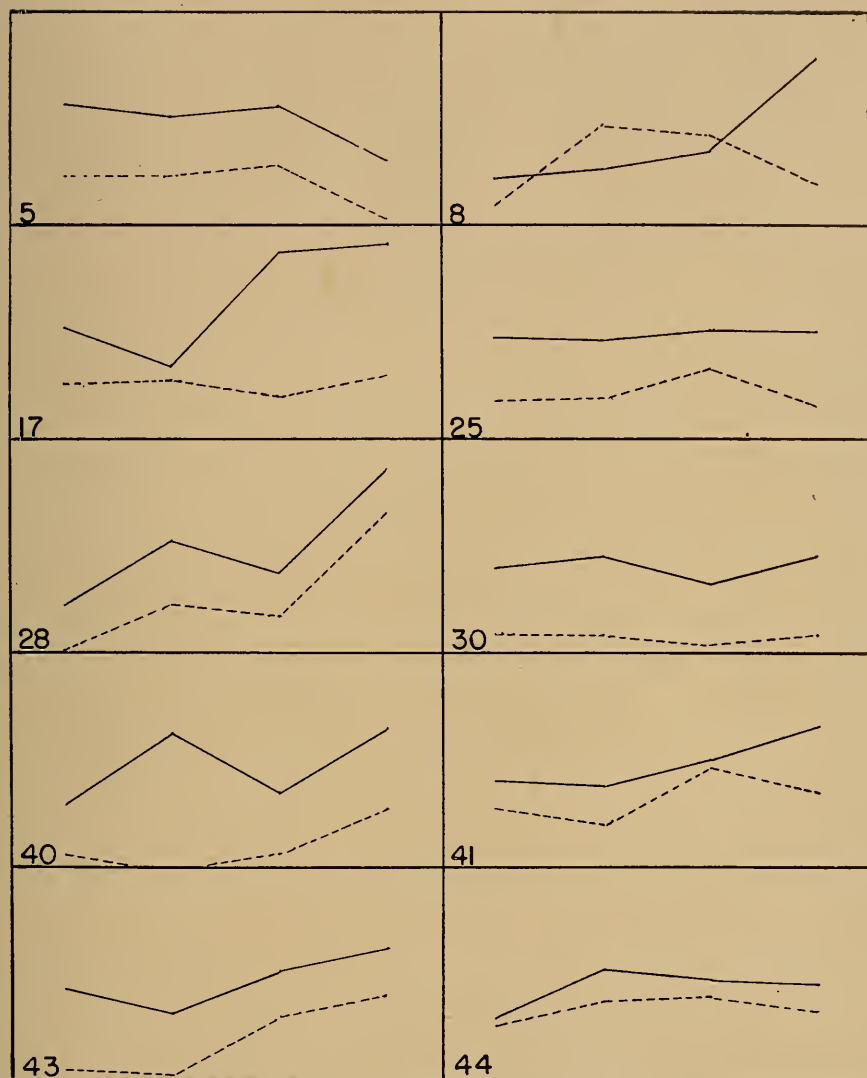


FIGURE 16. Mean length of ear in centimeters by generation of inbreeding when crossed by P39, for each variety. The broken line represents 1940 and the solid line 1941.

There is also the possibility that the rise from the second to the third generation is due to an increase in variability produced by selection of vigorous plants in the second generation. This trend was noticeable for height of the inbreds themselves, discussed previously.

As in the analysis of variance for yield and length of ear, the difference between years is highly significant as is the term for gen-

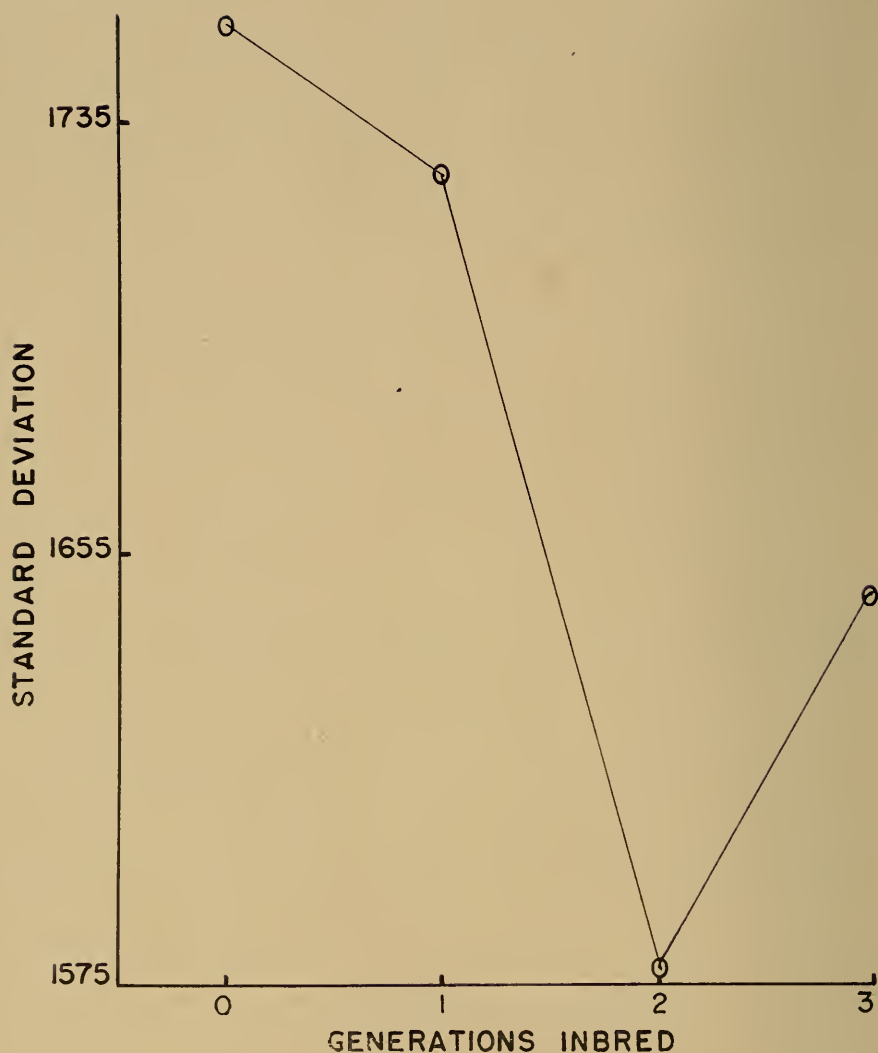


FIGURE 17. The standard deviation of mean length of ear by generation of inbreeding when crossed by P39, for all varieties and combining 1940 and 1941 results.

eration of inbreeding. The term for varieties is significant, showing that for all generations there was a significant difference between at least two varieties. The F value for the interaction variety  $\times$  generation is also significant.

Figure 18 shows the standard deviation plotted against generation of inbreeding for each of the ten varieties and in each of the years 1940 and 1941. In contrast to similar graphs for yield and mean

length of ear, there appears here to be much less correlation between the two years.

An analysis of covariance was computed for each year, using mean length of ear as the independent variate and the standard deviation in length as the dependent variate. For both years, the  $b$  was a very small minus number, indicating that the standard deviation was independent of mean length of ear.

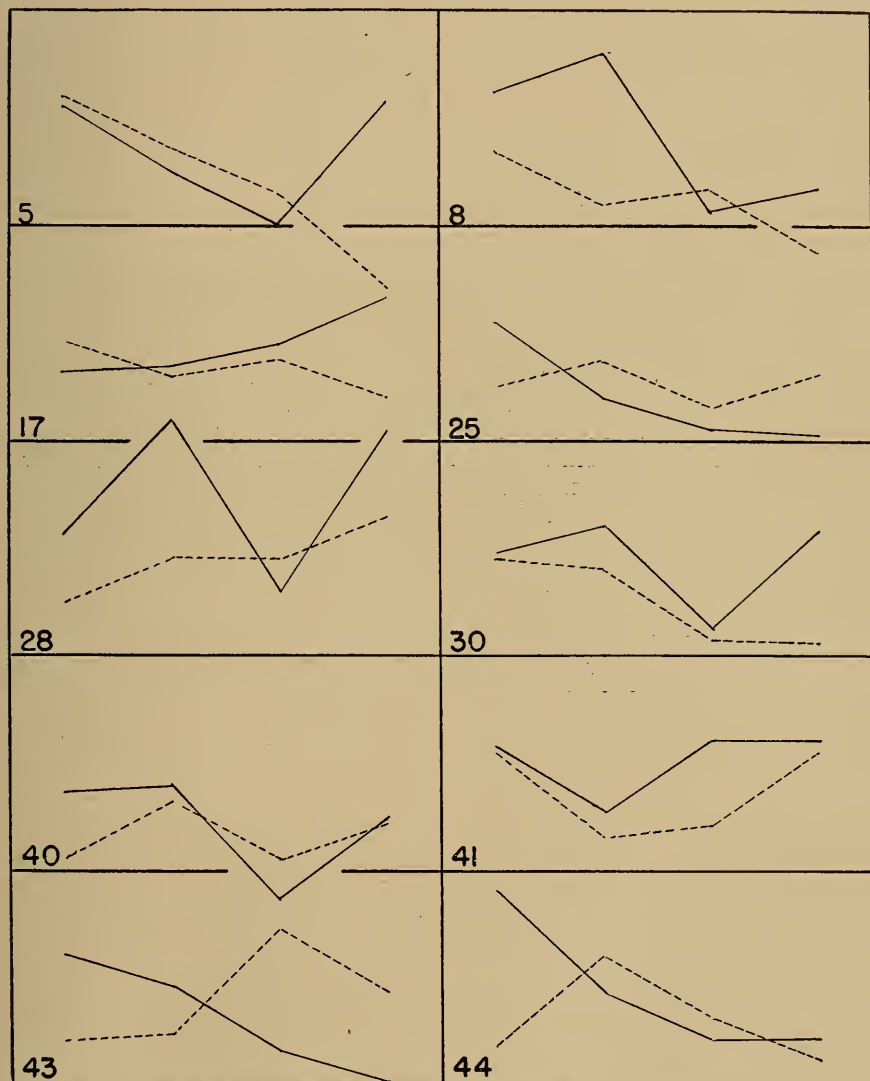


FIGURE 18. The standard deviation of mean length of ear by generation of inbreeding when crossed by P39, for each variety. The broken line represents 1940 and the solid line, 1941.



TABLE X. ANALYSIS OF VARIANCE FOR STANDARD DEVIATION LENGTH OF EAR.  
1940 and 1941

Source of Variation	DF	MS	F
Blks. + (Blks. $\times$ Years)	8	8.71	.65
Years	1	99.01	7.42**
Var.	9	35.00	2.62*
Var. $\times$ Years	9	11.23	.84
Var. $\times$ Blks. $\times$ Years	72	13.35	
Generations	3	64.35	5.18**
Linear	1	113.77	9.16**
Quadratic	1	24.50	1.97
Cubic	1	54.78	4.41*
Gen. $\times$ Years	3	30.79	2.48*
Gen. $\times$ Var.	27	23.85	1.92**
Gen. $\times$ Var. $\times$ Years	27	8.25	.66
Error	238	12.42	

## EFFECT OF EXPERIMENTAL RESULTS ON BREEDING PROCEDURE

The purpose of this investigation is to determine whether it is possible and advisable to test inbreds for combining ability in the early generations of inbreeding. Whereas, the common practice is to test inbreds after selfing at least three generations, would it be better to test in the second or first generation or even in the open-pollinated ear before inbreeding at all?

The problem of testing inbreds in the early generations of inbreeding resolves itself into two questions:

1. Is it possible to determine the potential inbreds with high combining ability in first or second generations of inbreeding and, if so, will the potential high combining ability persist when inbred further?

2. Is it practical to test in the early generations even if possible to do so?

The answers to these questions are not as easy as they might seem and, consequently, there has been some confused thinking on the whole problem. Jenkins (1935), obtained a positive answer to the first question and, on the basis of these results, answered the second in the positive also and advocated testing inbreds in the early generations for combining ability. Sprague and Bryan (1941) agreed with Jenkins and advocated rather strongly the testing of inbreds in the early generations, although they presented evidence to show segregation for combining ability in the  $F_3$  and  $F_4$  generations. In regard to the practicability of testing early or inbreeding further, they state the cost of the two systems would be about the same and that "this problem is a local one and must be decided by each worker on the basis of

local conditions". We disagree in regard to cost of early testing versus further inbreeding and, also, we believe the problem is *fundamental* and not local. In regard to testing versus further inbreeding, it is comparatively inexpensive to make hand pollinations and thus secure additional inbred generations. To test adequately as many as 100 corn hybrids requires considerable land and the test should be conducted in more than one season before results are conclusive. Even the most comprehensive and accurate tests yet devised will not provide sufficient data to enable a breeder to say a hybrid is definitely superior to all others in the trial or to say that this group of hybrids is definitely inferior and the inbreds used in making them should be discarded.

On the other hand, the cost of producing corn inbreds is comparatively small. To make 1,000 self pollinations would require not more than a week of a worker's time, probably much less. The cost of producing 1,000 inbreds would undoubtedly be less than running an adequate test on 100 lines in crosses, and the results are more definite. When the self pollinations are made, many inbreds will be obtained. Whereas, when the tests of early generations are concluded, there is still the problem of which ones to discard, in other words what proportion of all should be kept. Also, will the combining value of the inbreds persist? Here, the evidence is conflicting. The data of Jenkins (1935) showed that it will. However, the results reported here show no correlation for combining ability between the open pollinated ear and the third generation, or between the first and third, or second and third. On the basis of these data, we would recommend *not* to test for combining ability in the early generations of inbreeding. Clearly, more experimental evidence is needed on this subject and a project has been started to secure additional evidence from field corn inbreds. On the basis of all evidence now available, it seems best not to test for combining ability before an inbred has been selfed three times. Possibly further selfing would be better but we do know that testing after three generations of inbreeding has been successful in developing a number of sweet corn hybrids that have remained fairly constant and productive for several years. And that, from a breeder's standpoint, is the "proof of the pudding".

One factor overlooked in the advocacy of early testing is the bulk of experimental evidence by a considerable number of investigators that there is a correlation between the good inbreds and the higher yielding hybrids. This literature is reviewed in this bulletin. Most tests have shown that the inbreds classified as good on the basis of morphological characters, as well as the yield of the inbreds, will produce *proportionately* as many or more of the high yielding hybrids than all other inbreds in the same series. Since this is true, why be concerned whether good lines can be detected early and whether they will maintain their combining ability? We have all the information we need to advocate inbreeding first and testing only the good lines after some degree of homozygosity has been obtained. If



FIGURE 19. Ear of C30 (left), three strains of Purdue 39, ear of Long Island Beauty, ear of C31-1, C31-1  $\times$  C31-2, and C31-2. C31 was secured by crossing P39  $\times$  Long Island Beauty and backcrossing three times to P39. Note large ear of C31 in comparison with Purdue 39.

we can test only 100 lines or 50 lines, the policy should be to begin with 1,000 or 500 lines and then, before testing, discard all but 100 or 50. We will be discarding some good germ plasm, to be sure, but the ones left for testing will have proportionately as many high yielding hybrids as the whole group would if tested, and the inbreds selected will have sufficiently good agronomic characters to make seed production practicable. Every field corn breeder knows of excellent double crosses that are difficult to produce because one inbred is weak and maintained with difficulty. Seed production of a double cross is no stronger than its weakest inbred. By the policy of delayed testing of inbreds, such weak inbreds would be discarded before testing and considerable trouble saved.

Also, during the inbreeding process, the lines can be subjected to natural or artificial infections of bacterial wilt, smut, stalk and ear rots and any other diseases that are serious to the corn plant. By so doing, it will be possible to secure inbreds with resistance to the more important corn diseases. Inbreds can also be selected for morphological characters during the inbreeding process. This opportunity to select for good agronomic characters and for disease resistance in the inbreds is one of the best arguments, if more arguments are needed, for delaying the testing of inbreds until the third inbred generation.



There is some experimental evidence to show that a smaller inbred can produce a larger yield when crossed (Singleton, 1943A). The inbred C30, a reduced plant type of Purdue 39, produces, when crossed by C13 and C15, hybrids that are higher yielding than similar P39 crosses. Also, the hybrid,  $P39 \times C30$ , shows hybrid vigor over P39 (Singleton, 1943B). Jones (1944) has obtained heterosis in field corn reduced lines when crossed back on the line from which they arose. Outcrosses to other inbreds have not been tested. We are not prepared to state how general this type of reaction is but, until more evidence is obtained, it may be safe to assume this is a special, rather than the general, manifestation of hybrid vigor. Selection of the better lines would automatically eliminate such lines as C30 but we believe there is more to be gained by choosing the better lines for testing, than is to be lost by possibly discarding a few lines similar to C30. The line C30 is being used commercially in the production of Carmelcross C13.30. This is the only such line we know of in commercial use.

Another good reason for delayed testing for combining ability is that it is much easier to watch for mutations after an inbred is fairly homozygous. Mutation has largely been overlooked in all the discussions on maize breeding, probably because changes of this type are so infrequent. However, we know that variation does occur for visible characters and, also, for combining ability, as has been pointed out. The C30 arose from Purdue 39, presumably by mutation. Also, there are several distinct strains of Purdue 39 that have arisen, most likely by mutation. Some are the result of crossing and backcrossing but several distinct strains have arisen by small changes from the original P39. These inbreds behave differently when crossed by P51 and some, perhaps all, of the distinct strains will give hybrid vigor when crossed together. The most striking example of this is in the line C31 which is a P39 type with a larger plant and ear. C31 was secured from a cross of P39 and Long Island Beauty, backcrossed three times to P39. Ears of the different types are shown in Figure 19. After sib pollinating for one generation, the C31 lines were then inbred for three more generations before they were crossed. The pedigrees of the two lines when crossed were C31-1a C and C31-2b B, showing that 31-1 was selfed three generations after the first sib pollination while 31-2 was sib pollinated twice and selfed twice. We might expect some hybrid vigor in a cross of this type but not much since both lines were derived from the Long Island Beauty-P39 cross that had been backcrossed three times to P39. Since each backcross is more than equivalent to a generation of selfing, these lines had been inbred three times before the two sub lines were split off. It is the opinion of most plant breeders that three backcrosses are sufficient to bring the backcrossed line almost back to the original, and some advocate not more than two backcrosses.

When 31-1 was crossed by 31-2, it was quite surprising to find hybrid vigor almost equivalent to that obtained when unrelated lines



FIGURE 20. C31-1, (left) C31-1  $\times$  C31-2 (four ears) and C31-2. Inbreds very closely related, come from the same stock which should have been fairly homozygous before lines were separated.

are crossed (Figure 20). The explanation for such a phenomenon is somewhat obscure. There is the possibility that the original C31 line was sufficiently heterozygous to allow segregation of factors to give the remarkable hybrid vigor found. There is also the possibility that the lines have mutated or diverged in different directions in the nature postulated by East (1936). Further work is necessary to decide which of these postulates is correct. Since there is a possibility that mutation is the explanation, it seems to us that maize breeders should be on the alert for mutations, not only in visible morphological characters, but also for factors affecting combining ability. This is a large task if any number of inbreds is to be watched closely. But it should be done if we are going to make intelligent use of all the facts available in a breeding program. It is our opinion that commercial producers of hybrid sweet corn seed could more profitably spend their time testing established inbreds for combining ability and thus improve the hybrids already in existence than in conducting rather elaborate breeding programs searching for new and distinctive hybrids.

From all the evidence now at hand, it seems to us the proper procedure in maize breeding is to inbreed at least three generations, then discard all poor and unproductive lines before testing for combining ability. The most economical and satisfactory means of securing a



number of inbreds for testing is by the hill method or non-individual pedigree method proposed by Jones and Singleton (1934). It seems to us this method has not been utilized to its fullest extent. Most maize breeders still keep pedigrees of each individual inbred clear back to the first selfed generation. Why? The answer may be for sentimental reasons. Since it is desired to test a number of inbreds that have been inbred three times, why not get those inbreds by the most direct method? This can be done by the non-individual pedigree method. By this method, seed from the original open pollinated ears is put in individual packets of paper or in individual coin envelopes (Figure 21). Only one pedigree is kept for the whole lot whether there be 100, 500 or 1,000. The paper packets are dropped in different hills or, if several hills of each line are desired, the coin envelopes are laid out in the field and the seed planted in such a manner that each line is kept separate. This can be done by skipping a hill or two after each line is planted. Two pollinations are made in each line (more if desired) but *only one* is saved to continue the line. Thus, each hand pollinated ear represents an individual inbred line and, hence, no individual labeling is necessary. The process is then repeated for each succeeding generation of selfing. As long as *only one* ear of each line is saved, then no labeling of individual lines is neces-



FIGURE 21. 322 Spanish Gold Inbreds ready for planting. Each "pellet" is dropped in a separate hill. One hand-pollinated ear is saved from each line. One label serves for whole lot of 322 lines during inbreeding.

sary. Everyone, we believe, will agree that it is better to select *among* rather than *within* lines. The hill or non-individual pedigree method of inbreeding deserves much wider usage, since it seems to be the most efficient method of producing a large number of inbred lines.

## SUMMARY

The purpose of this investigation is to determine the earliest generation of inbreeding in which it is possible and practical to select for factors for combining ability. This involves how early combining ability can be detected, how stable it is after detected, and whether it is practical to test for combining ability in the very early generations of inbreeding.

For this study, 48 open pollinated ears of Whipple's Early Yellow were selected and grown first in 1935. One self-pollination was made in each line and pollen from the plant selfed was put on Purdue 39, a productive uniform sweet corn inbred. In 1936, the seed from the selfed ears was grown again and the process repeated. Preliminary observation rows of the hybrids P39  $\times$  Whipple lines O P, were grown in 1936 and, in 1937, these were grown again along with the P39  $\times$  Whipple lines selfed once. In 1936 and 1937, hybrids were selected on the basis of yield, general vigor, appearance and uniformity of the hybrid ears. In 1938, the inbreds grown were reduced to ten and four different generations of each were grown and studied for uniformity. The four generations studied were O P, selfed once (A), selfed twice (B), selfed three times (C). Characters studied were days to tassel and silk, with standard deviation of each, plant height with standard deviation, and average number of tillers. In all lines, tasseling was earlier than silking, although, in some lines, the difference between tasseling and silking was greater than others. The lines became slightly later upon inbreeding. The standard deviation for tasseling did not decrease upon inbreeding. If anything, it increased, showing the lines were as variable for tasseling at the end as at the beginning. There was a slight decrease in the standard deviation for date of silking, showing a very slight increase in uniformity.

The height of all lines decreased rapidly for the first and second generations of inbreeding. There was a rise in plant height from the second to third generation of selfing, probably due to selection of thrifty plants in the second inbred generation. The standard deviation for height decreased as inbreeding increased, showing the lines were becoming more uniform for plant height. This was true for most lines, even when height increased in the third generation, probably indicating the vigorous lines selected in the second generation were not extremely heterozygous. All lines became fixed for tillering fairly early in the inbreeding process and remained relatively stable thereafter.

The ten Whipple lines in four different generations of inbreeding were crossed by Purdue 39. The crosses of the four different degrees of inbreeding within the ten varieties by Purdue 39 were grown in both 1940 and 1941 with good agreement between years for all the characters studied. Considering all varieties, selection within inbred lines was effective in raising combining ability significantly from the open pollinated ear to the third inbred generation. A large part of this increase in yield is due to the increase in the mean length of ear which shows a steady increment from generation to generation when outcrossed by P39. The standard deviation of the mean length of ear shows in general a downward trend. The standard deviation of length of ear is not related in this case to the magnitude of the length of ear. Since there was no correlation between yield of the open pollinated generation and the third or between the first and third, or second and third, it was not possible to detect combining ability in generations earlier than the third generation of selfing.

Even when possible to select for combining ability in generations earlier than the third, it is probably inadvisable for two reasons: (1) lines are still segregating or changing for combining ability, and (2) the difficulty of testing properly a large number of lines in hybrid combination is so great that it is advisable to inbreed further and reduce the lines in number before testing. This point is fully discussed here.

Since it seems best to inbreed for at least three generations before testing, the inbreds should be secured by the most direct method with the least amount of labeling of individual lines. This can be done most efficiently by the non-individual pedigree method of inbreeding, a method that deserves much wider usage.

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